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EFFECTS OF HYPERTHERMIA ON THE ULTRASTRUCTURE OF SPONTANEOUS MOUSE MAMMARY TUMORS WITH REFERENCE TO VIRAL DYSMORPHOGENESIS

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ABSTRACT. The mechanisms of cell death by hyperthermia were investigated in the spontaneous mammary tumors of the Strong A strain of mice. Circulating hot-water in a latex bag was used to apply a local heat dose of 46°C for 1 hour to the tumors in anaesthetized mice. The tumors were surgically removed from the mice under anesthesia at various times after heat treatment and studied by electron microscopy. Cytoplasmic swelling and condensation of nuclear chromatin occurred 5 minutes after heat treatment. Degenerative changes then became progressively more pronounced at 24, 48, and 72 hours after heat treatment. This included disorganization of the cytoplasm and loss of organelles, a marked increase in the number and size of lysosomes, the disruption of plasma membranes, the loss of nuclear membranes, nucleoli, and the fragmentation of condensed chromatin. There was also infiltration by granulocytes, and bundles of collagen fibrils into the tumor tissue. The mouse mammary tumor virus particles in the heated tumor cells were deformed and turned into amorphous masses. Our findings suggest that heat induced degenerative changes in the spontaneous mouse mammary tumors occurs through a combination of mechanisms including mitochondrial damage, rupture of cell membranes, damage to nuclei, and a marked increase in lysosomal activity, the latter playing the primary role in the hyperthermic killing of malignant cells.

Keywords: Hyperthermia, plasma membranes, lysosomes, mitochondria and chromatin

Hyperthermia is a very old form of cancer treatment in man. The first recorded use of hyperthermia in cancer treatment appeared in the writings of Indian physician Ramajama (2000 B.C.) who observed the palliative effects of applying hot irons to superficial tumors (Storm & Morton 1983; Coffey *et al.* 2006; Horseman & Overgaard 2007). Hyperthermia has been used alone or in combination with radiation and chemotherapy for many years (Dewey *et al.* 1973; Horsman *et al.* 2001; Halika *et al.* 2007). However, despite the promising results, it has not been widely accepted because of limitations in clinical application (Hildebrandt *et al.* 2002; Horseman & Overgaard 2007; Pennacchioli *et al.* 2009).

It has been shown that certain tumors in mice, dog, and man can be destroyed by immersing affected areas in a constant temperature water bath at a 42 to 46°C range for 7½ to 60 minutes without damaging the surrounding normal tissue. Destructive effects of heat began at 42°C, at which temperature it took several hours to damage the tumor tissue. Neuroblastoma in an infant immersed in hot water bath at 46–47°C temperature for 67 minutes was completely destroyed (Crile 1961; 1962). Cavaliere *et al.* (1967) found that hyperthermia in the 42–45°C temperature range caused irreversible damage to Novicoff hepatoma cells but not to normal rat liver cells, suggesting that tumor cells were more sensitive to heat than normal cells. Hyperthermia delivered by radiofrequency electromagnetic fields to EM-6 tumors implanted in mice showed an almost 50% cure rate of the tumors heated for 5 minutes at 44°C temperature (Marmor *et al.* 1977). In recent years, new techniques have been used for the application of hyperthermia. Xie and Sun (2006) demonstrated that hyperthermia induced by an

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electrothermal needle in combination with electrochemical therapy was a potentially effective procedure in treating solid malignant tumors in mice. An attractive approach for the treatment of deeply seated malignant tumors with heated magnetic iron particles is receiving considerable attention. In this procedure, magnetic iron nanoparticles are delivered to the tumor and then heated by an external magnetic field set at frequencies to generate the required temperature. However, one of the serious drawbacks of this procedure is the unwanted heating of the surrounding normal tissue (Ito *et al.* 2006; Thiesen & Jordan 2008). More recently, thermal treatment of the lumpectomy cavity with a hot balloon has been developed and tested in the goat mammary gland as an adjunct to surgery of breast malignant tumors (Alvarado *et al*. 2009). Only a few histological and ultrastructural studies have been carried out on the effects of hyperthermia on malignant tumors. Light and electron microscopic studies were carried out on mouse mammary carcinoma HB implanted in the flank of C3H mice treated with high-frequency diathermy at 41–43°C temperature range for 30 minutes (Overgaard & Overgaard 1972; Overgaard 1976). The effects of hyperthermia were investigated by light and electron microscopy in EMT-6 neoplasms implanted in adult C3H strain mice using radiofrequency electromagnetic fields at 44°C temperature for 30 minutes (Fajardo *et al.* 1980). However, the ultrastructural changes in spontaneous mouse mammary tumors exposed to hot-water hyperthermia have not yet been investigated. The mouse mammary tumor virus (MMTV) is known to cause mammary carcinoma in C3H and Strong A strains of mice. It is found in large amounts in lactating mammary tissue and thus readily transferred to suckling mice in which the incidence of developing mammary carcinoma is high (Bishop 1978; Jawetz *et al.* 1987).

The aim of the present study was to elucidate the mechanism by which hyperthermia applied through a hot-water bag induces subcellular destructive changes including MMTV in the spontaneous mammary tumors in the Strong A inbred strain of mice.

METHODS

Adult females of the Strong A strain of mice which have been inbred for many generations and have a high frequency of spontaneous mammary tumors, were used in this study. The animals were obtained from the colony of mice maintained in the animal room facility of the Biology Department, Ball State University, Muncie, IN. The animals were treated according to an approved Ball State University Animal Care and Use Committee protocol. Female mice bearing a spontaneous tumor about 10 mm in diameter were anaesthetized with an intra-peritoneal injection of 40 mg/kg Nembutal. The tumors located in the anterior thoracic wall were then exposed to hyperthermia through a thin latex bag that contained circulating hot-water, according to the method designed by Walker (1980). This method is a form of water heating in which intra-tumormal temperature rises because of conduction, rapidly achieving uniform temperature distribution (Walker, 1980). Tap water in a 2-gallon polycarbonate jar or reservoir was heated to 47°C using a TU-15 TEMPUNIT thermoregulator (Bailey Instruments, Saddle Brook, NJ). Circulating hot water from the reservoir was delivered to the heating unit, through silicon tubing using a bellows-type metering pump (Bailey Instruments, Saddle Brook, NJ). A separatory funnel was used between the metering pump and the heating unit in order to provide regular flow of water to the heating unit. After circulating through the heating unit and the water bag, hot water was returned through silicon tubing to the reservoir. The heating unit proper was constructed from Plexiglas tubing and had an inlet and outlet. To obtain a good temperature distribution, holes were drilled at an angle to direct the flow of water as an eddy around the tumor. Horizon Stimula contraceptive sheaths (Akwell Industries, Skokie, IL) were used as water bags (Fig. 1). Good thermal contact between the conducting bag membrane and the tumor was achieved by using chemically inert K–Y Jelly. The tumors were heated at 46°C temperature for 1 hour. During the experiments, body core temperature was monitored rectally by a Bailey rectal probe for mice (Bailey Instruments, Saddle Brook, NJ). The tumor temperature was measured with a Bailey digital thermocouple thermometer microprobe embedded in the tip of a fine needle for placement within the tumor for measuring intratumoral temperature. The unheated and heated tumors were surgically removed under anesthesia at 5 and 20 minutes, and at 24, 48, and 72 hours after

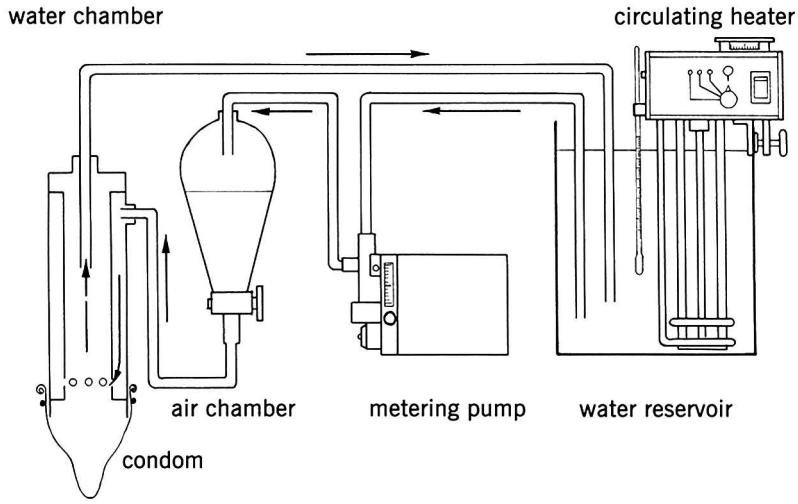

Figure 1.—Diagrammatic representation of the heating apparatus used in this study.

heat treatment. After surgical removal of the tumors, the mice were returned to the colony for further breeding. The experimental animals exhibited no systemic side effects after the heat treatment. After removal, the tumors were processed for electron microscopy. The tumor tissue was fixed at room temperature in 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in an ethanol series to propylene oxide, and embedded in poly/BED resin (Polysciences, Warrington, PA). Polymerization was carried out at 60°C overnight. Thin sections were cut on a Porter-Blum MT2 ultra microtome, stained with uranyl acetate and lead citrate and examined with a Hitachi HU 11A transmission electron microscope.

RESULTS

Temperature Measurements.—Tap water was heated in the reservoir to a temperature of 47°C. After circulating through the tubing the temperature recorded was 46°C at the tip of the heating water bag. There was thus a loss of 1°C, as the hot water circulated from the reservoir to the water bag. The surface of the tumors thus received a heat dose of 46°C. The body core temperature of the anaesthetized mice monitored by a rectal probe recorded an average reading of 32°C. The rectal temperature rose 1°C during the initial few minutes of heating, but returned to near normal after 1 hour. During heat application, an average intratumoral temperature of 40°C was recorded. The tumor tissue received a heat dose of 8°C higher than the mouse's body core temperature.

Electron Microscopy.—The electron micrographs, of the untreated mouse mammary tumor cells were bounded by delicate plasma membranes and displayed large nuclei containing uniformly distributed euchromatin and conspicuous marginal heterochromatin abutting the nuclear membrane and a prominent nucleolus (Figs. 2A, 2B). The cytoplasm contained small mitochondria, abundant ribosomes, and a few lysosomes, but rough endoplasmic reticulum and Golgi complexes were sparse (Figs. 2A, 2C). In addition, numerous virus particles were present around vacuoles and dispersed in the cytoplasm of tumor cells. A few virus particles were also present in the lumina of the vacuoles. (Figs. 2A, 2D).

In the 1 hour heat-treated mammary tumor cells degenerative ultrastructural changes occurred in the nuclei and the cytoplasm, which became progressively more pronounced with an increase in time after heat treatment. At 5 minutes following heat treatment, the tumor cells displayed moderate condensation of the nuclear chromatin and swelling of the cytoplasm due to fluid accumulation in the round spaces (Fig. 3A). The plasma membranes of the tumor cells remained intact (Fig. 3B).

Twenty minutes after heat treatment, there was an increase in condensation of the nuclear chromatin accompanied by shrinkage of the

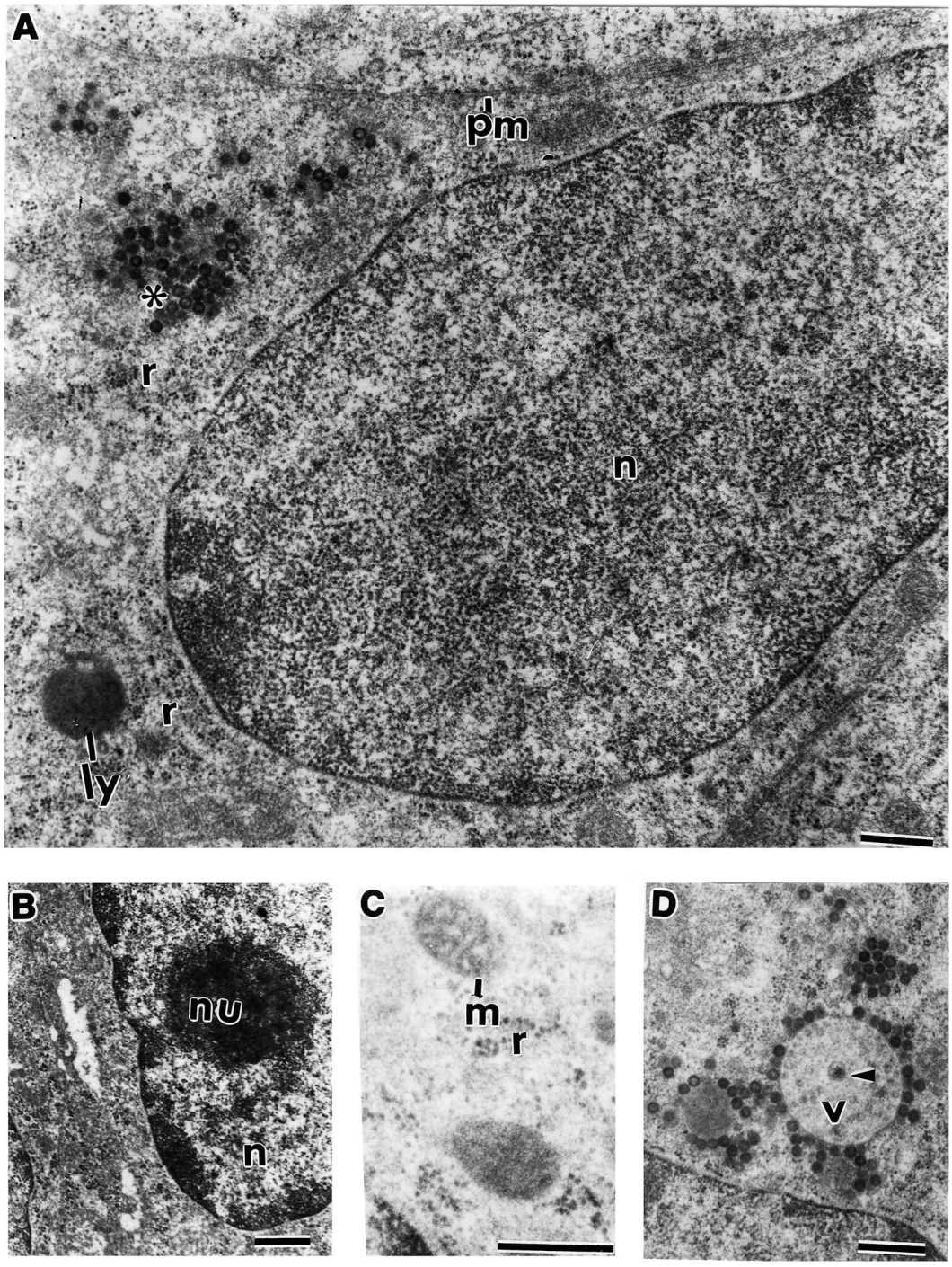

Figure 2.—Electron micrographs of untreated tumor cells. **(A)** A tumor cell showing a delicate plasma membrane (pm) and nucleus (n) containing euchromatin and conspicuous marginal heterochromatin. The cytoplasm contains a few lysosomes (ly), abundant ribosomes (r), and a cluster of virus particles (*). **(B)** Part of an untreated cell nucleus (n) displaying a prominent nucleolus (nu). **(C)** Cytoplasm of an untreated tumor cell showing mitochondria (m) and ribosomes (r). **(D)** Untreated tumor cell cytoplasm displaying many immature virus particles in it and around a vacuole (v). A mature virus particle (arrowhead) is also seen in the lumen of the vacuole (v). Scale bar: 0.5 μm A–D.

cytoplasm. The cytoplasm displayed small electron-opaque mitochondria, large vacuoles (Fig. 3C), and altered electron-lucent virus particles (Fig. inset 3C). Many electron-dense lysosomes were found in the tumor cell's disorganized cytoplasm (Fig. 3D). Clusters of collagen fibrils infiltrated into the tumor mass (Fig. 3E).

The destructive changes in the 1 hour heat-treated tumor cells became progressively more pronounced at 24 and 48 hours after heat treatment. At 24 hours the nuclei of the tumor cells appeared small and shrunken with disrupted nuclear membranes, markedly condensed chromatin and obscured nucleoli. The cytoplasm became disorganized and mitochondria were dilated with a loss of cristae (Fig. 4A). The intracellular virus particles became electron-lucent (Fig. 4A inset). Large lysosomes with heterogeneous contents including virus particles, multivesicular bodies, and many electron-dense lysosomes were found in the cytoplasm of the tumor cells (Figs. 4B, 4C). Large lipid droplets were also seen in the cytoplasm of the tumor cells (Fig. 4D).

At 48 hours after heat treatment the tumor cells displayed small shrunken degenerating nuclei with dark markedly condensed chromatin and a wide clear perinuclear space occasionally containing altered virus particles were found in the tumor cells. (Fig. 5A). The cytoplasm became highly vacuolated with numerous virus particles in it and around the vacuoles. There were also seen a few apparently intact virus particles and remnants of damaged particles in the lumina of vacuoles (Fig. 5B). The cytoplasm also displayed multivesicular bodies and many electron-dense lysosomes but other organelles were not seen (Fig. 5C). The tumor mass was infiltrated by collagen fibrils (Fig. 5C) and eosinophilic granulocytes containing many banded specific granules (Fig. 5D).

At 72 hours after heat treatment, the tumor cells displayed pronounced destructive changes. The plasma membranes were lost, and the cytoplasm was completely disorganized, and most of the organelles were destroyed with the exception of lysosomes. The small, dark pyknotic nuclei had disrupted nuclear membranes and were devoid of nucleoli (Fig. 6A). The cytoplasm contained many electron-dense lysosomes (Fig. 6B) and large lamellar lysosomes (Fig. 6C). A few altered virus particles were found in the cytoplasm (Fig. 6D). The tumor tissue was infiltrated by abundant collagen fibrils (Fig. 6E).

In the untreated tumor cells, the mouse mammary tumor virus (MMTV) occurred in two morphologically distinct forms: A type and B type. The A type 'immature' particles about 87nm in diameter consisted of a ring-shaped nucleoid surrounded by a membrane and a submembranous electron-dense layer or shell. These particles mainly occurred in the cytoplasm and around the vacuoles but a few were found in the lumina of the vacuoles (Figs. 2A, 2D, 7A, inset 7A). The A type particles after acquiring a membranous envelope were budded off into the extracellular space as B type particles (Fig. 7C). The B type 'mature' particles about 98nm in diameter consisted of an eccentric round nucleoid covered by a loose membrane and a thin submembranous layer (Fig. 7C inset). The loose, flaccid membraneous envelopes apparently caused heterogeneous shapes and size of B type particles in the extracellular space of the thin sections (Fig. 7C). The B type particles occurred mainly in the extracellular spaces, but a few particles were also found in the lumina of the vacuoles (Figs. 2D, 7C).

In the 1 hour heat-treated tumor cells 48 hours after heat treatment, at higher magnifications the MMTV particles in the cytoplasm and those in the vacuoles were morphologically altered and converted into amorphous masses. Some particles remained intact (Fig. 7B). Since the plasma membranes of the tumors were disrupted by heat treatment, budding of the virus particles was inhibited. The virus particles seen in the extracellular space were apparently released prior to heat treatment of the tumors (Fig. 7D).

DISCUSSION

This study has shown that destructive ultrastructural changes occur in the spontaneous mouse mammary tumors exposed to hot-water hyperthermia. The changes started as early as 5 minutes after the tumor was heat-treated for 1 hour at 46°C. The tumor morphology became progressively more pronounced within the days after heat treatment.

The heat-treated tumor cells displayed degenerative changes in the cytoplasm, plasma membranes, and the nuclei. The virus particles in the heat-treated tumors were also altered.

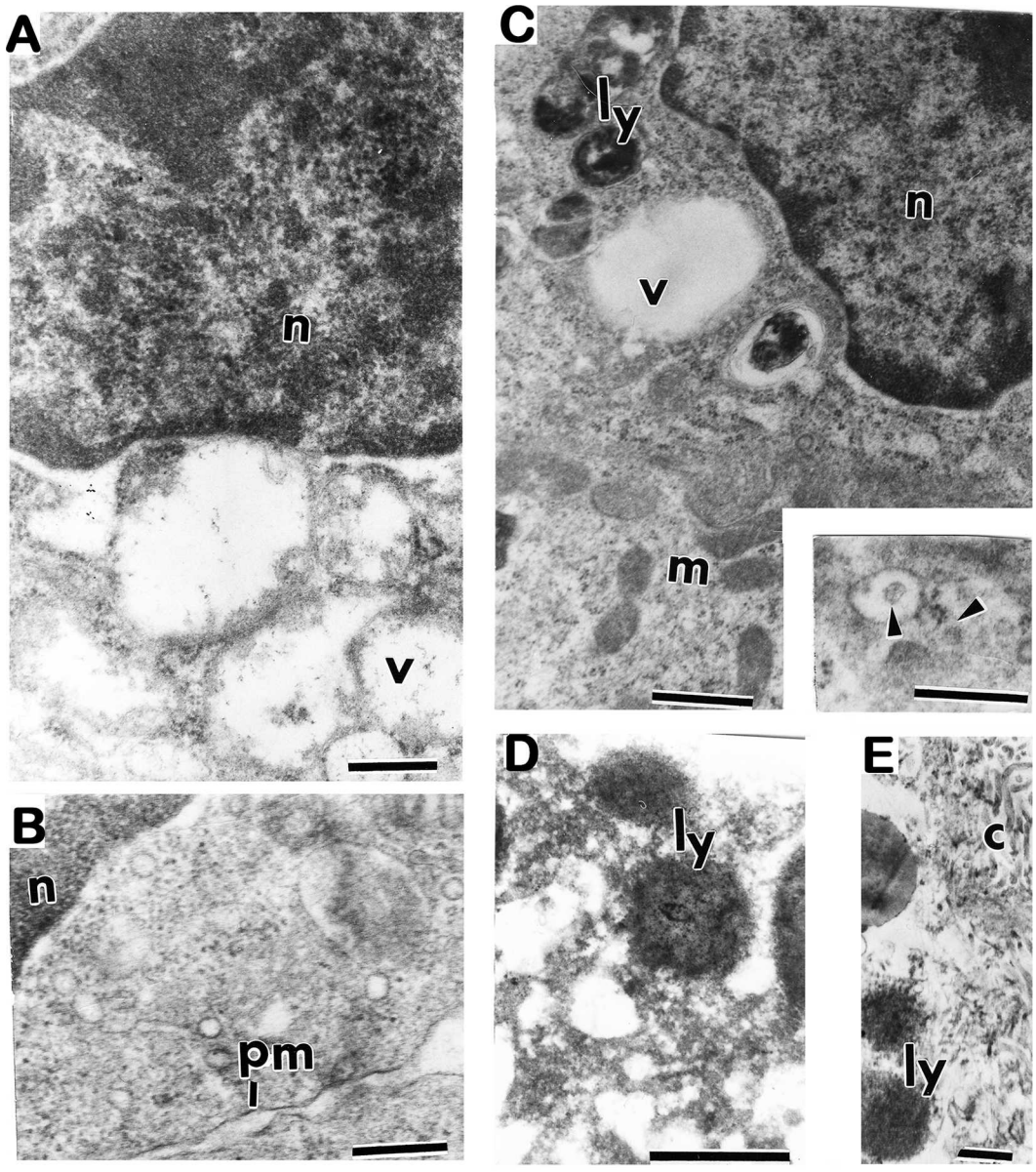

Figure 3.—Electron Micrographs of 1 hour heat-treated tumors 5 minutes and 20 minutes after heat treatment. **(A)** A portion of a heat-treated tumor cell 5 minutes after hear treatment showing swelling of cytoplasm and condensation of nuclear chromatin, a nucleolus (n), and vacuole (v). **(B)** A segment of a tumor cell 5 minutes after heat treatment showing an intact plasma membrane (pm) and nucleus (n). **(C)** Part of a tumor cell 20 minutes after heat treatment showing a nucleus (n) with increased condensation of chromatin. The cytoplasm displays many lysosomes (ly), electron-opaque mitochondria (m) and large vacuoles (v). The inset shows altered electron-lucent virus particles (arrowhead) in the tumor cell cytoplasm. **(D)** Part of a tumor cell 20 minutes after heat treatment showing electron-dense lysosomes (ly) in the disorganized cytoplasm. **(E)** Bundles of collagen fibrils (c) and lysosomes (ly) in the tumor tissue. Scale bar: 0.5 μm A, B, inset C; 1 μm C–E.

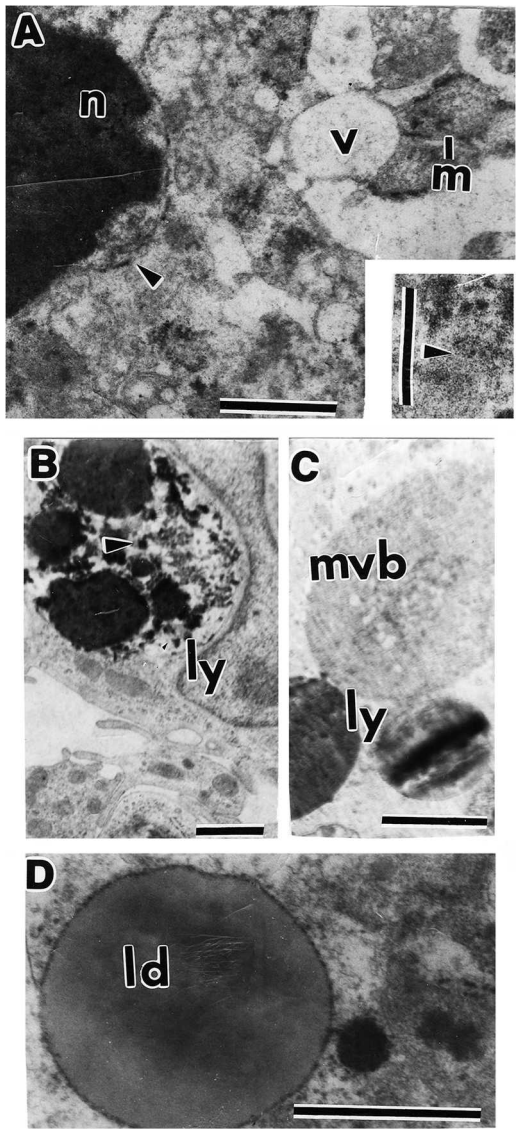

Figure 4.—Electron micrographs of 1 hour heat-treated tumor cells 24 hours after heat treatment. **(A)** Part of a tumor cell showing small pyknotic nucleus (n) with broken nuclear membrane (arrowhead) The cytoplasm contains vacuoles (v) and mitochondria (m) devoid of cristae. Inset shows altered intracytoplasmic virus particles. **(B)** A large lysosome (ly) containing heterogeneous contents including a virus particle (arrowhead) in a tumor cell's cytoplasm. **(C)** A multivesicular body (mvb) and lysosomes (ly) in a tumor cell's cytoplasm. **(D)** A large lipid droplet (ld) in the cytoplasm of a tumor cell. Scale bar: 1μm A–D and A inset.

The first changes observed 5 minutes after heat treatment were cytoplasmic swelling and condensation of the nuclear chromatin followed by increased shrinkage and disorganization of the cytoplasm resulting in the loss of endoplasmic reticulum ribosomes, Golgi complexes, and disruption of mitochondria. After 20 minutes, 24, 48, and 72 hours after heat treatment, observations indicated the continuation of degenerative changes. There was a marked increase in the number and size of lysosomes in the cytoplasm of heat-treated tumor cells, which peaked at 24 hours after heat treatment. Similar findings have been reported by Overgaard (1976) in implanted mouse mammary tumors treated with high-frequency diathermy at 41–43°C temperature range for 30 minutes. Damage to mitochondria by heat apparently inhibits aerobic metabolism. Anaerobic glycolysis, which in turn becomes important, tends to increase acidity in the heated energy-deficient tumor cells, thus making them more susceptible to damage by lysosomal acid hydrolases (Overgaard 1976; Fajardo *et al.* 1980).

The disruption of the plasma membranes of the heat-treated cells observed in this study apparently changes the permeability allowing materials to flow in and out of the cells causing swelling and disorganization of the cytoplasm. It has been proposed that cellular heat injury is associated with a dramatic increase in membrane permeability to Na^+ and K^+ between intracellular milieu and extracellular spaces. A change in the stability of lipoproteins and enzymes, which maintain structural integrity of the plasma membrane, is thought to cause cellular heat injury and ultimately cell death (Fajardo *et al.* 1980; Overgaard 1976).

Loss of the rough endoplasmic reticulum, ribosomes, and nucleoli apparently inhibits cellular protein synthesis and polymerization of RNA and DNA in heat-treated tumor cells (Bowler *et al.* 1973; Fajardo *et al.* 1980). The presence of large lipid droplets in the heat-treated tumors probably represents accumulation of saturated fatty acids (Bowler *et al.* 1973), but their role in heat injury remains unclear.

The nuclear changes in the heat-treated tumor cells include condensation of the chromatin, loss of nucleoli, and disruption of nuclear membranes, followed by fragmentation

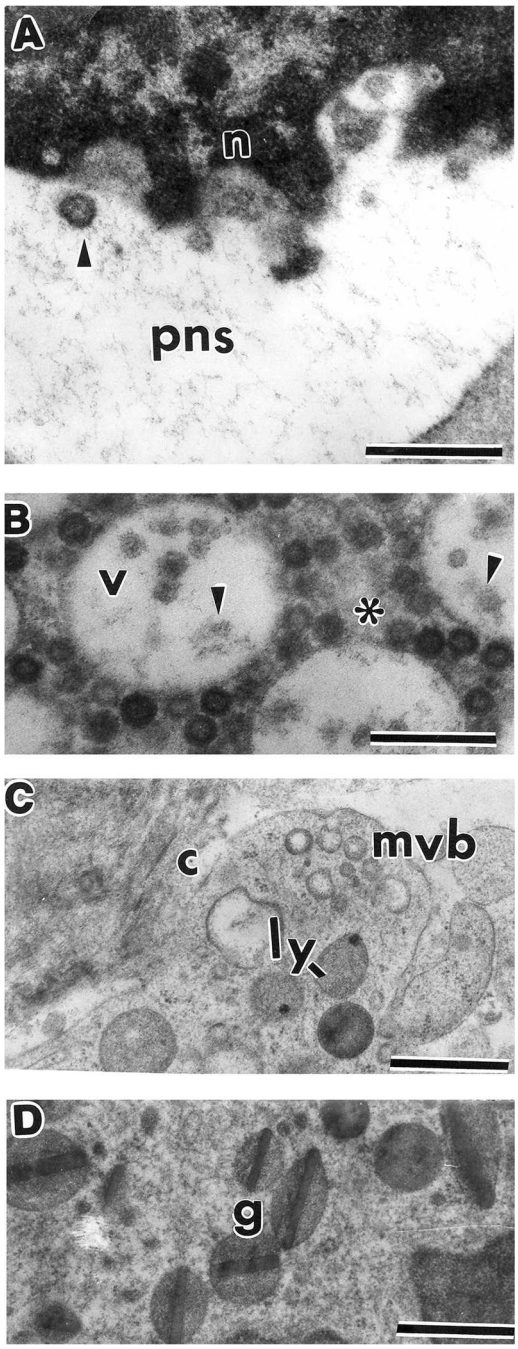

Figure 5.—Electron micrographs of 1 hour heat-treated tumor cells 48 hours after heat treatment. **(A)** Small pyknotic and degenerating nucleus (n) of a tumor cell surrounded by a large perinuclear space (pns) containing an altered virus particle (arrowhead). **(B)** Highly vacuolated cytoplasm of a tumor cell containing numerous immature virus particles (*) around vacuoles (v). A few mature virus particles and remnants of damaged virus particles (arrowheads) are seen in the vacuoles. **(C)** A multivesicular body (mvb), lysosomes (ly) and infiltrated collagen fibrils (c) in the cytoplasm of a tumor cell. **(D)** A portion of an eosinophilic granulocyte that infiltrated into the tumor containing many banded specific granules (g). Scale bar: 0.5μm A, B; 1μm C, D.

of nuclear chromatin. These findings clearly indicate severe heat damage to the nuclei of the tumor cells. It has been demonstrated that hyperthermia induces unfolding of the nuclear matrix and the subsequent changes in the binding of the specific proteins to the matrix, which may play an important role in hyperthermic killing of the tumor cells (Roti Roti *et al*. 1998; Coffey *et al*. 2006) The marked increase in infiltration of granulocytes, and bundles of collagen fibrils into the tumors 48 and 72 hours after heat treatment, suggests that remnants of tumor cells destroyed by heat are being phagocytosed and replaced with fibrosis (Alvarado *et al*. 2009). The heated tumors, if not surgically removed, would most likely become completely filled with fibrous tissue and eventually fall off (Crile, 1961).

The mammary adenocarcinoma in certain strains of mice is caused by mouse mammary tumor virus, (MMTV), which is an RNA retrovirus. It occurs in two forms: A type intracellular particles consisting of a ring-shaped nucleoid surrounded by a membrane and submembranous layer or shell and B type extracellular particles consisting of an eccentric round nucleoid covered by a loose membrane. In our study, MMTV particles resembled those described by other investigators (Bernhard >*et al*. 1955; Jawetz *et al*. 1987 Lyons & Moore 1975) except that A type particles displayed a prominent submembranous shell which resembled that of the immature particles of the human immunodeficiency virus (HIV) (Nermut 1994). The MMTV particles like those of other retroviruses apparently enter the mammary cells by adsorption, and after multiplying within the cells new particles are released by budding into the extracellular spaces. The presence of MMTV particles in vacuoles suggests that in addition to adsorption, the virus particles also enter the mammary cells by phagocytosis

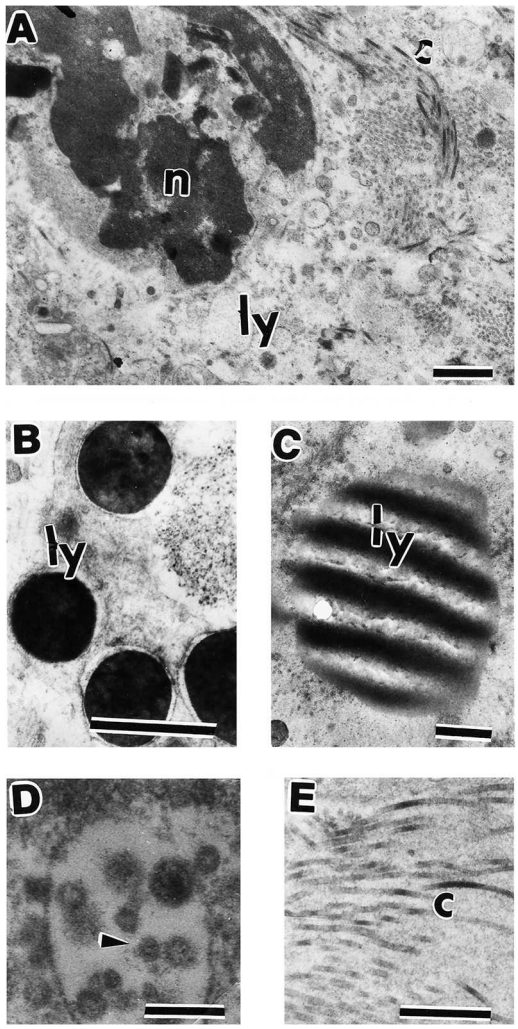

Figure 6.—Electron micrographs of 1 hour heat-treated tumor cells, 72 hours after heat treatment. **(A)** Small shrunken nucleus (n) of a tumor cell with extremely condensed chromatin and lost nuclear membrane. The surrounding degenerated cytoplasm contains small lysosomes (ly) and infiltrated collagen fibrils (c). **(B)** Dense lysosomes (ly) in the cytoplasm of a tumor cell. **(C)** A large lamellar lysosome (ly) in a tumor cell's cytoplasm. **(D)** Altered virus particles (arrowhead) in the cytoplasm of a tumor cell. **(E)** A field of tumor tissue showing abundant collagen fibrils (c). Scale bar: 1μm A–E.

(Morgan *et al*. 1969; Murray *et al*. 2002). It is thought that the vacuoles containing virus particles fuse with lysosomes. The acidity of the lysosomes causes the outer viral membranes to fuse with the membranes of the lysosomes causing the release of the nucleocapsids into the cytoplasm without being hydrolyzed by lysosomal enzymes (Simmons *et al*. 1982). In the heat-treated tumor cells, the structure of MMTV particles was altered. At higher magnifications, 48 hours after heat treatment the virus particles in the cytoplasm and vacuoles were deformed turning into amorphous masses. The viral RNA is synthesized in the nuclei of infected cells (Bishop 1978). The degenerative changes that occurred in the nuclei of heat-treated tumor cells, apparently inhibited RNA synthesis and consequently impeded the formation of new MMTV particles. The budding of the deformed virus particles was further inhibited by disruption of the plasma membranes in the heated tumor cells. Some intracellular virus particles which appeared structurally intact in heated tumor cells, were most likely functionally inactive. This view is supported by the observation that human warts which are circumscribed lesions caused by *Verruca, vulgaris* virus, when exposed to hot-water at 45–48°C temperature range for 1–1.5 hours, were cured and completely disappeared, since the virus apparently became inactive and decomposed following heat treatment (LoCricchio 1962).

This study has shown that hot-water hyperthermia causes profound degenerative changes in the cytoplasmic elements and destruction of MMTV particles, disruption of plasma and nuclear membranes, loss of nucleoli and nuclear membranes, condensation and fragmentation of nuclear chromatin of the mouse mammary tumor cells. Marked increase in the number and size of lysosomes in the heated tumor cells supports the hypothesis of Overgaard (1976) that increased lysosomal activity with the release of hydrolytic enzymes is the primary cellular reaction to hyperthermia. It is likely that all of these mechanisms play a role in hyperthermic killing of malignant tumor cells; but the marked increase in lysosomal activity is of prime importance. Our findings strongly suggest that hyperthermia delivered through a hot-water bag can be safely used to destroy certain human malignant tumors, including cervical cancer and head and neck squamous cell carcinomas.

ACKNOWLEDGMENTS

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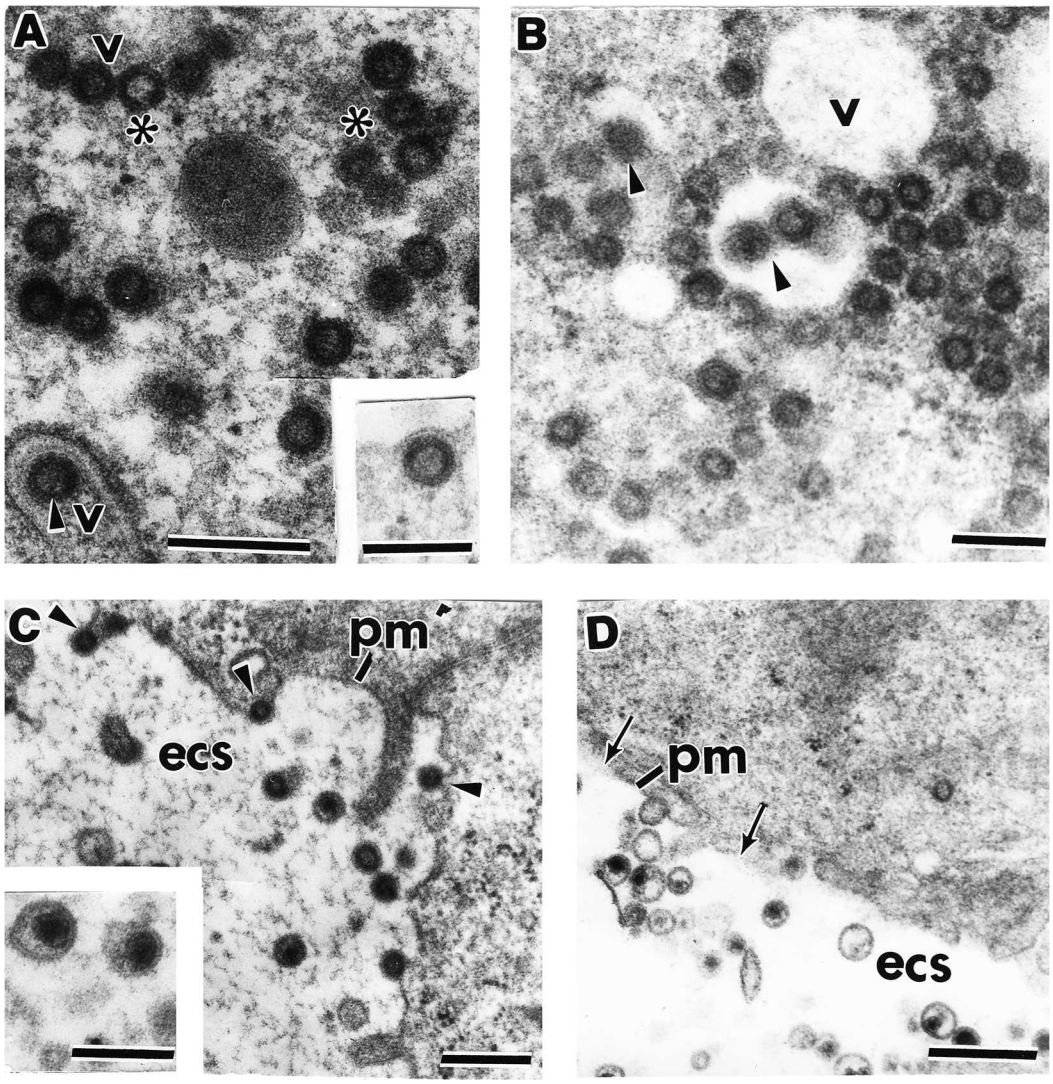

Figure 7.—An electron micrograph showing MMTV particles in the tumor cells. **(A)** Untreated tumor cell showing numerous A type virus particles (*) in the cytoplasm, around vacuoles (v) and a virus particle (arrowhead) in the lumen of a vacuole (v). Inset shows one A type virus particle at higher magnification. **(B)** Tumor cell heat-treated for 1 hour, 48 hours after treatment, showing numerous A type virus particles in the cytoplasm and vacuoles (v) structurally altered and turned into amorphous masses (arrowheads). A few virus particles remain intact. **(C)** The peripheral part of an untreated tumor cell showing virus particles budding (arrowheads) from the plasma membrane (pm) into the extracellular space (ecs). Inset shows mature B type virus particles at higher magnification. **(D)** Tumor cell 1 hour heat-treated, 48 hours after heat treatment displaying the plasma membrane (pm) ruptured (arrows) and absence of virus budding. Virus particles seen in the extracellular space (ecs) were apparently released from the infected tumor cell prior to heating. Scale bar: 0.25μm A–D, and insets A, C.

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LITERATURE CITED

Alvarado, R., B. Mahon, C. Valadez, M. Caufield & S. Wadhwani, et al. 2009. Thermal ablation of goat mammary gland as a model for post-lumpectomy treatment of breast cancer: preliminary observations. International Journal of Hyperthermia 25:47–55.

Bernhard, W., A. Bauer, M. Guerin & C. Oberling. 1955. Etude au microscope electronique corpuscules diaspect virusal dans des epitheliom memaires de la souris. Bulletin du Cancer. 42:163–178.

Bishop, J.M. 1978. Retroviruses. Annual Review of Biochemistry 47:35–88.

Bowler, K., C.J. Duncan, R.T. Gladwell & T.F. Davidson. 1973. Cellular heat injury. Comparative Biochemistry and Physiology 45:441–450.

Cavaliere, R., E.C. Ciocatto, B.C. Giovanella, C. Heidelberger & R.O. Johnson, et al. 1967. Selective heat sensitivity of cancer cells. Cancer Research. 20:1351–1381.

Coffey, D.S., R.H. Getzenberg & T.L. DeWeese. 2006. Hyperthermia biology and cancer therapies: A hypothesis for the ''Lance Armstrong effect.'' Journal of the American Medical Association 296:445–448.

Crile, G. 1961. Heat as an adjunct to the treatment of cancer. Cleveland Clinic Quarterly 28:75–89.

Crile, G. 1962. Selective destruction of cancers after exposure to heat. Annuals of Surgery 156:404–407.

Dewey, W.C., D. Thrall & E.L. Gilette. 1977. Hyperthermia and radiation-a selective thermal effect on chronically hypoxic tumor cells in vivo. International Journal of Radiation Oncology, Biology and Physics 2:99–103.

Fajardo, L.F., B. Egbert, J. Marmor & G. Hahn. 1980. Effects of hyperthermia in a malignant tumor. Cancer. 45:613–623.

Hahn, G.M. 1982. Hyperthermia and Cancer Plenum Press, New York.

Halicka, H.D., B. Ardelt, K. Shogen & Z. Darzynkiewicz. 2007. Mild hyperthermia predisposes tumor cells to undergo apoptosis upon treatment with onconase. International Journal of Oncology 30:841–847.

Hildebrandt, B., P. Wust, O. Ahlers, A. Dieing & G. Sreenivasa, et al. 2002. The cellular and molecular basis of hyperthermia: Critical Reviews in Oncology/Hematology. 43:33–56.

Horsman, M.R., R. Murata & J. Overgaard. 2001. Improving local tumor control by combining vascular targeting drugs, mild hyperthermia and radiation. Acta Oncologica 40:497–503.

Horsman, M.R. & J. Overgaard. 2007. Hyperthermia: a potent enhancer of radiotherapy. Clinical Oncology. 19:418–426.

Ito, A., H. Honda & T. Kobayashi. 2006. Cancer immunotherapy based on intracellular hyperthermia using magnetic nanoparticles: a novel concept of ''heat-ontrolled necrosis'' with heat shock protein expression. Cancer Immunology and Immunotherapy 55:320–328.

Jawetz, E., J.L. Melnick & E.A. Adelberg. 1987. Review of Medical Microbiology seventeenth edition. Appleton & Lang, Los Altos.

LoCricchio, J. & J.R. Haserick. 1962. Hot-water treatment for warts. Cleveland Clinic Quarterly 29:156–161.

Lyons, M.J. & D.H. Moore. 1965. Isolation of the mouse mammary tumor virus: chemical and morphological studies. Journal of the National Cancer Institute 35:549–565.

Marmor, J.B., N. Hahn & G.M. Hahn. 1977. Tumor cure and cell survival after localized radiofrequency heating. Cancer Research 34:879–883.

Morgan, C., H.S. Rosenkranz & B. Mednis. 1969. And development of viruses as observed in the electron microscope x. entery and uncoating of adenovirus. Journal of Virology 4:777–796.

Murray, P.R., K.S. Rosenthal, G.S. Kobayashi & M.A. Pfaller. 2002. Medical Microbiology, fourth edition. Mosby, St. Louis.

Nermut, M.V. 1994. Electron microscopy of the human immunodeficiency virus. Microscopy and Analysis. USA 8:7–9.

Overgaard, K. & J. Overgaard. 1972. Investigations on the possibility of a thermic tumor Therapy I. Short wave treatment of a transplanted isologous mouse mammary carcincoma. European Journal of Cancer. 8:65–78.

Overgaard, J. 1976. Ultrastructure of a mouse mammary carcinoma exposed to hyperthermia *in vivo*. Cancer Research. 36:983–995.

Pennacchioli, E., M. Flore & A. Gronchi. 2009. Hyperthermia as an adjunctive treatment for soft-tissue sarcoma. Expert Review of Anticancer Therapy 9:191–210.

Roti Roti, J.L., H.H. Kampinga, R.S. Malyapa, W.D. Wright & R.P. Wanderwaal, et al. 1998. Nuclear matrix as a target for hyperthermic killing of cancer cells. Cell Stress Chaperones 3:245–255.

Simmons, K., H. Garoff & A. Helenius. 1982. How an animal virus gets into and out of its host cell. Scientific American 246:58–66.

Storm, F.K. & D.L. Morton. 1983. Local hyperthermia in the treatment of cancer. CA-A Cancer Journal for Clinicians. 33:44–56.

Thiesen, B. & A. Jordan. 2008. Clinical applications of magnetic nanoparticles for hyperthermia. International Journal of Hyperthermia. 24:467–474.

Walker, A. 1980. A simple non-stressful method of heating experimental tumors in unanesthetized

mice. Physiology of Medical Biology 25:365–368.

Xie, L. & C. Sun. 2006. A new local ablation for solid malignant tumors: Antitumor effect of hyperthermal and electrochemical therapy on transplantable mouse cancer. International Journal of Hyperthermia 22:607–612.

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